Chapter

Discovery of Hepatitis C Virus: Nobel Prize in Physiology and Medicine 2020

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Abstract

Scientists were successful in discovering Hepatitis A and B, but there is another virus which has a long incubation period, many people are asymptomatic and cause adverse effects. Three scientists Harvey J Alter, Michael Houghton and Charles M Rice who have contributed their work in discovering a non-A, non-B hepatitis virus called Hepatitis C. Hepatitis is a disorder associated with the functioning hepatic cells in the liver. The person infected with Hepatitis C will have poor functioning of liver, vomiting, fatigue, jaundice and appetite. In this paper, I am going to explain about the Hepatitis C virus, and the work was done by three scientists and various research around it.

Keywords: Hepatitis C, Post-transfusion, non-A non-B hepatitis, Gene expression, Antigen antibody reaction

1. Introduction

Hepatitis C virus is a blood-borne pathogen. The person infected with this virus has defects in the functioning of the liver and blood. The progress of the virus in the human body is slow acting. The incubation period varies from person to person, it is about 2–3 months [1]. Hepatitis C is associated with chronic hepatitis which means inflammation of the liver and may also lead to liver failure sometimes cancer called Hepatocellular carcinoma [2]. According to the World Health Organization, it was estimated that there are about 70 million of the total world population infected with the Hepatitis C virus [3, 4]. If the treatment is delayed, the disease will progress and cause liver cirrhosis and hepatocellular carcinoma [5]. Hepatitis C is causing 400 000 deaths annually [4].

2. Discovery of hepatitis A and B

In 400 B.C., Hippocrates called hepatitis infection as 'Epidemic Jaundice' and told that "The bile contained in the liver is full of phlegm and blood, and erupts.. Such an eruption, the patient soon raves, becomes angry, talks nonsense and barks like a dog" [6]. During the second world war, the infection to the liver was thought of infection by several viruses and called it 'Viral Hepatitis'. After that, in 1947,

British hepatologist F.O. MacCallum has classified viral hepatitis into Hepatitis A which is Epidemic hepatitis and Hepatitis B which is serum hepatitis [7].

Baruch Blumberg (1925–2011) was a geneticist at National Institute of Health in Bethesda who is working on human disease susceptibility. He collected blood samples of people from many places in the world to study inherited diseases and susceptibility [7]. He found an unfamiliar reaction taking place in the serum of a hemophilic patient who needs blood and an Australian aborigine who is a donor. He initially thought that he discovered a new lipoprotein. After that, in the serum of a hemophilic patient, he could find detection of a new antigen, he called that as 'Australian-antigen' [7, 8]. In 1967, Blumberg linked the Australian-antigen with viral hepatitis, and in 1968, Alfred Price used Immuno-electrophoretic technique to explain that serum antigen that Blumberg discovered was related to hepatitis and called it as Serum hepatitis antigen. Later, both Australian-antigen and Serum hepatitis antigen were confirmed that these are viral particles. Blumberg performed several serological tests using chimpanzees to confirm the antigens are of Hepatitis B virus. In 1976, Blumberg got Nobel Prize Physiology and Medicine [9]. At that time, it was impossible to identify who are carriers of diseases and who are healthy donors, the effect of disease on a person is silent and progressive [4].

3. Discovery of hepatitis C

3.1 Harvey J Alter

Along with Blumberg, there is another person who also contributed his work in discovering Hepatitis B is Harvey J Alter. Alter also worked at National Institute of Health in Bethesda [10]. In the 1970s, people started studying the relationship between blood donors infected with Hepatitis B and post-transfusion hepatitis [8]. While they were studying about this, Alter found out that, though Hepatitis B positive donors prevented from donating blood, he found that blood transfused people were still infected with other 'Hepatitis related infections' [11]. Alter came across a patient who had a mild form of the disease and later that patient had Hepatitis associated diseases after a long incubation period. Based on this, he proposed that there may be two different viruses causing 'post-transfusion hepatitis' [11]. In 1975, Feinstone, Purcell and other scientists tested patients who are non-B hepatitis and found that Hepatitis A is not causing the disorders [12, 13].

The blood transfusion of non-B hepatitis was spreading to more numbers of people. They were sure the infection was not because of Hepatitis A or B, then came up with a term called 'non-A, non-B hepatitis' (NANBH) [12, 13]. Alter and his colleagues were clear that NANBH is responsible for post-transfusion hepatitis, but they were unable to show what NANBH is? Since there is no tool to diagnose NANBH, many people got affected by blood transfusion. The only animal model which is susceptible to NANBH is chimpanzees, Tabor et al. [14] have infected chimpanzees to study the hepatocyte infection and agents causing the disease. They have taken plasma from NANBH people and infected chimpanzees, and they found cirrhosis and hepatocellular carcinoma disorders in animal [4]. After several experiments, Alter and his colleagues found that NANBH has essential lipids which are enveloped around the virus which are different from Hepatitis B [15, 16]. Alter did not give conclusive results to state the causative agent is causing post-transfusion hepatitis.

3.2 Michael Houghton

In 1982, Houghton worked at Chiron Corporation and came up with molecular methods called cDNA library. Houghton and his colleagues infected chimpanzees with NANDH virus and have taken plasma from them, that plasma they have centrifuged to get a pellet of virus and they have extracted the nucleic acid from it. They have denatured the nucleic acid because they do not know whether it is DNA or RNA. After denature, they synthesized the cDNA [17]. They transduced the cDNA to a bacterial vector using a bacteriophage λ gt11 strain, the method is called transduction [18]. The bacterial vector undergoes translation to display cDNA-encoded polypeptides. They also looked for whether similar antigen that is expressed in the serum of NANBH patients by using screening techniques. If an antigen is found in the body, the immune system will generate antibodies against it. They have considered those patients as sources of viral antibodies, they have taken plasma by centrifuging blood of NANBH patients [18]. The bacterial vector has expressed the cDNA proteins, and by introducing plasma of the patient to the bacterial colony, the antibodies in the plasma will bind to polypeptides of bacteria [4]. Based on this idea of Molecular Biology and Immunology, they performed several screenings of the above experiment about 10^6 and found there is one colony that did not match human or chimpanzee DNA sequence, it matched with the sequence of a virus family called Flaviviridae [4]. They named it as cDNA clone 5–1-1 and named it as Hepatitis C virus (**Figure 1**) [17– 20].

Houghton and his colleagues have immediately taken this knowledge further. They have collected suspected blood samples from Alter and performed the above experiment on those samples. They found all the blood samples they have tested are positive Hepatitis C. Using this diagnostic technique, donors were tested blood samples before transfusion which decreased the number of hepatitis cases [4]. But, Houghton has not evidently proved that Hepatitis C is only the causative agent or a mix of viruses causing disorder?

3.3 Charles M Rice

To find out what is actually causing chronic liver cirrhosis, two scientists Kunitada Shimontohno and Charles Rice came up with a new experiment. Blight and Rice [21], they have sequenced the viral genome and found that it is positive RNA strand about 96000 nucleotides, the RNA undergoes direct translation to form proteins, the primary transcription process is eliminated. The viral genome is a long

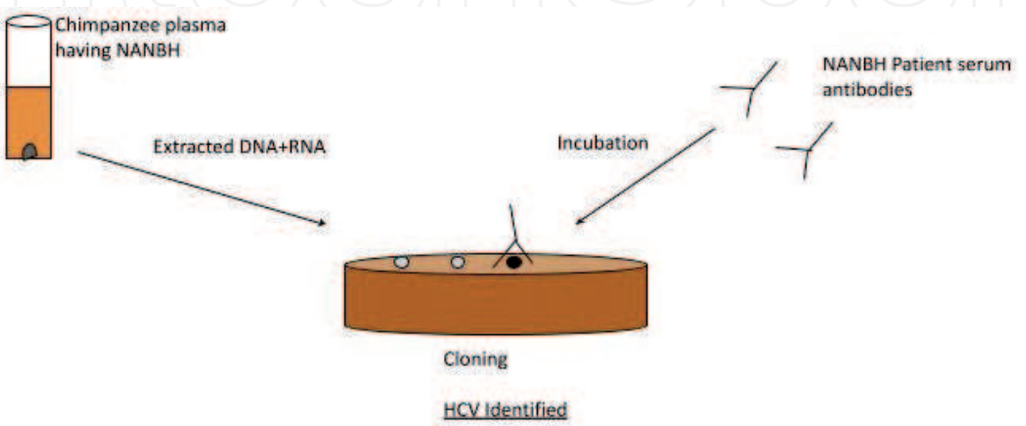

Figure 1.
Summary of Houghton work.

open reading frame (ORF), different types of proteins are translated from one ORF which has several translation initiation and termination codons [21].

They have found that there is a non-coding region at the 3'and 5′ ends of the viral RNA genome which is responsible for replication of the virus [21, 22]. Kolykhalov et al. [23] have constructed a viral genome which has conserved 3′ region at 5′ nontranslated region (5' NTR) and rest in long ORF (**Figure 2**). That genome gene they have injected to the chimpanzee liver to check the viral replication, but unfortunately the experiment failed, they did not find new viruses in the blood. While finding reasons for failure of experiment, they came across that during replication, mutations are common in the viral genome. To eliminate the mutations, they have engineered a few new sequences with silent markers. With all new sequences, they have created a new repaired conserved 3′ region (**Figure 3**) [4, 23, 24]. They repeated the above experiment with a newly engineered genome and the experiment worked resulting in chimpanzees having liver cirrhosis and hepatocellular carcinoma. Based on this, Rice gave the conclusion that only Hepatitis C virus alone causes hepatitis, there no other causative agent involved.

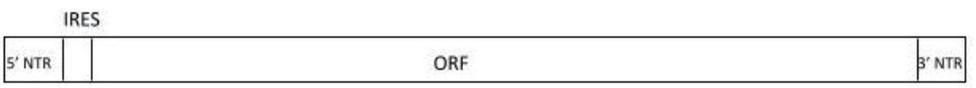

Figure 2.
Viral genome with conserved 3' region.

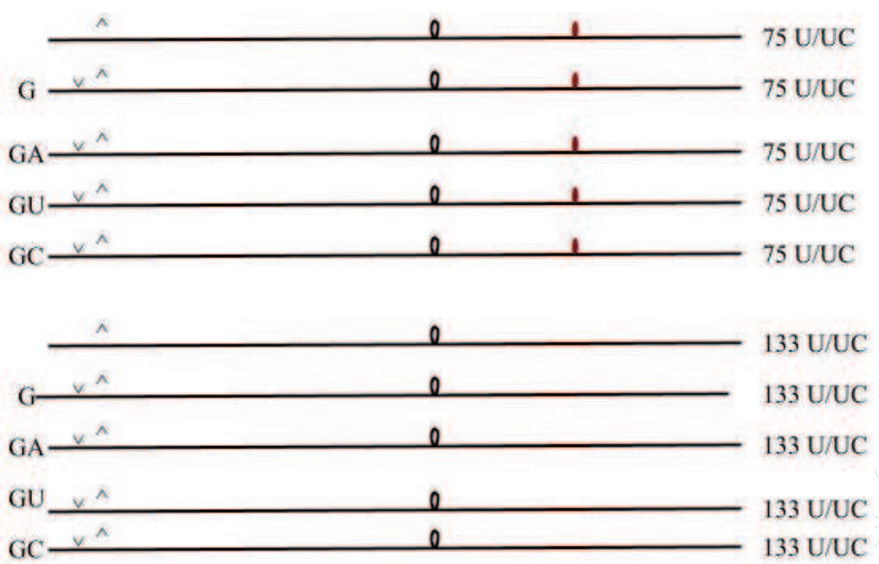

Figure 3.
Repaired conserved 3' region Genomes [23].

4. Mode of infection and diseases

Virus Type	Family	Genetic material	Disorders	Disorders
Hepatitis A	Picornaviridae	RNA	Contaminated food and water	Abdominal pain, nausea, fatigue
Hepatitis B	Hepadna	DNA	Blood transfusion	Liver failure, jaundice
Hepatitis C	Flaviviridae	RNA	Blood transfusion	Inflammation of liver and hepatocellular carcinoma

5. Molecular mechanisms of replication

Structure of Virus: Hepatitis C is a single-stranded RNA virus belonging to the family of Flaviviridae and there are seven genotypes (gt 1–7) and 67 subtypes which states genetic diversity is high [5]. The size of the virus is about 56–65 nm in diameter and the viral core about 45 nm. In the viral envelope, there are viral spikes which are formed by E1 and E2 glycoprotein heterodimers. Viral membranes consist of several lipoproteins those are low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and apolipoproteins (Apo) which are A1, B, C (**Figure 4**) [5].

Viral Genome: As described above, viral genomes contain 96000 nucleotides and one ORF with the coding region of 3010 to 3033 nucleotides and 5′ and 3′ ends have non-translational (NTR) regions. The translation of viral RNA takes place in the endoplasmic reticulum of hepatic cells in the liver which is initiated by the IRES region which is adjacent to 5'NTR (**Figure 3**). The translation results in the formation of three structural proteins which are core, E1 and E2 and seven non-structural proteins which are p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B. The structural proteins form viral components and non-structural proteins regulate viral growth and replication (**Figure 5**) [5].

Viral Cycle: When a person has blood transfusion from the Hepatitis C infected person, the virus reaches to the liver cell and binds to the lipoviral receptor proteins and the whole virus is engulfed into the cell by a process called clathrin-mediated endocytosis. The virus reaches the endoplasmic reticulum and releases its RNA. The ORF of RNA is translated to form structural and non-structural proteins.

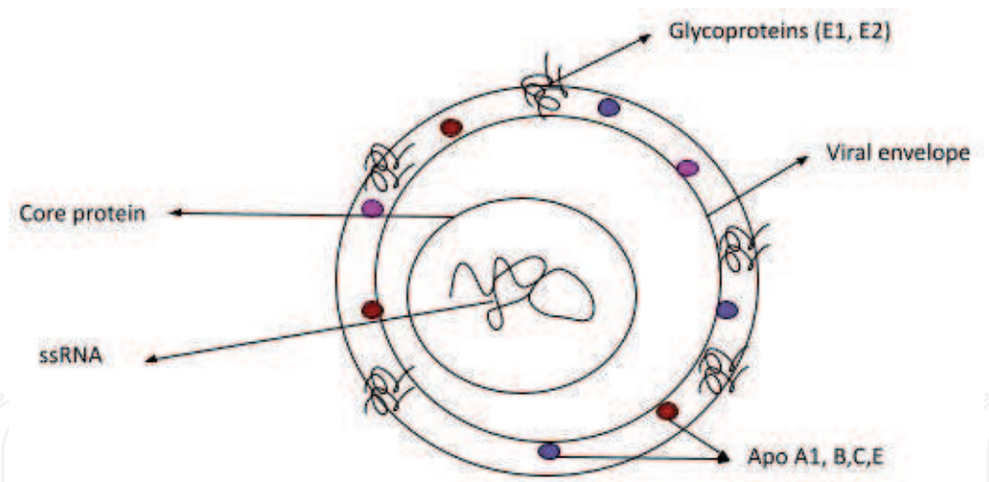

Figure 4.
Structure of hepatitis C .

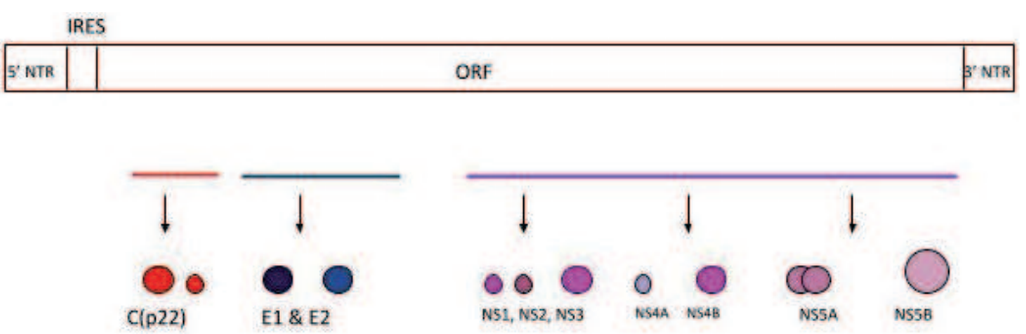

Figure 5.
Viral genome expression [5].

As I mentioned, the structural proteins form viral components and non-structural proteins regulate viral growth and replication using cellular components. The viral components and replicated RNA fragments reach the Golgi apparatus and unite to form mature viruses. The formed viruses enter the blood by bursting the liver cell. One single entry produces millions of viruses that cause liver dysfunction by bursting hepatic cells [5].

6. Research: a way to discovery of vaccine

Phosphatidylinositol 4-kinase III α (PI4KA) is a hepatic cellular protein which converts phosphatidylinositol to phosphatidylinositol 4-phosphate (PI4P) [25]. This protein has several roles in the viral replication and growth in an infected cell. PI4KA interacts with structural proteins in shaping the virus and also interaction with the non-structural protein of NS5B will accumulate the essential cellular material for viral growth [26]. When the Phosphatidylinositol 4-kinase III?? is knocked down, the replication and production of viral components are affected. Harak et al. [26] have done an in-vitro gene knockdown method to inhibit viral growth. Sarhan et al. [27] have also done similar experiments. They found other proteins called GSK3 α and β interacting with viral NS5A. The GSK3 α and β phosphorylates the NS5A. The phosphorylation of NS5A results in multiple functions such as viral maturation and release. If the GSK3 α and β genes are knocked down, the viral maturation and release is inhibited [27].

When any foreign particles enter the body, our immune system will identify that antigen. The human immune system has B cells, T cells and Natural killer cells play essential roles in detecting antigens. Hepatitis C virus has E2 glycoprotein in the core. CD81 markers which are present on B-cells will interact with E2 glycoprotein [28]. The binding of E2 and CD81, B-cells release serum antibodies to neutralize the viral activity. Research around Molecular Biology and Immunology will increase the chances of discovering the vaccine. Research is the stepping stone to discovering new things in science.

7. Diagnosis and treatment

When Hepatitis C is infected, the majority of the people are asymptomatic. The incubation period varies from person to person. In order to detect the virus, there are diagnostic tests to be performed. There are two ways to direct the virus, one is an indirect method based on antibodies production and direct method based on viral detection. In the indirect method, a person's blood sample will be taken which consist of serum, blood and plasma. To that blood, recombinant viral proteins core, NS3 and NS4 antigen are added. Along with recombinant proteins, colloidal gold labeled protein A is added. If the antibodies bind to antigens, the recombinant protein generates reddish-purple lines. This screening test will reveal that antibodies are present. To confirm the person infected with Hepatitis C, Recombinant Immunoblot Assay (RIBA) is antibody specific test which will direct anti-hepatitis C antibodies [29]. In the direct method diagnosis, Reverse-transcriptase polymerase chain reaction (RT-PCR) is performed which directly gives confirmatory results whether the virus is present or not [29].

The current work going on Hepatitis C is discovering a vaccine. To cure Hepatitis C, there is no vaccine. If the disorder is in advanced stages, the person needs liver transplantation. If Hepatitis C is directed at early stages like at chronic hepatitis stage, there are antiviral drug treatments which cure disorder to some extent. These

antiviral drugs interfere with viral replication and maturation [3]. There are several classes of drugs which interfere with viral growth. The nonstructural 3/4A inhibitor drugs Boceprevir and telaprevir interfere with NS3/4 A proteins to inhibit the viral protein formation. Nonstructural 5A inhibitors like Ledipasvir, ombitasvir, daclatasvir etc., will interfere with NS5A protein which plays an important role in viral replication and assembly of viral particles. Nonstructural 5B inhibitors like sofosbuvir interfere with NS5B which synthesizes viral RNA [3]. Treating patients with antiviral drugs will inhibit viral progress in the body. These drug targets cure the disease if the disease is at an early stage.

8. Conclusion

Alter, Houghton, Rice and their colleagues have contributed their work to the world of science. They have come up with new molecular and immunological techniques to detect the presence of viruses. Alter and his colleagues discovered an Australian antigen and it was non-A, non-B hepatitis (NANBH). He introduced a model organism chimpanzees to study the disease post-transfusion hepatitis. Houghton and his colleagues have brought Molecular Biology and Immunology together and diagnosed NANBH and named it as Hepatitis C virus. Rice and his colleagues sequenced the viral genome and explained its properties of replication and gene expression. He discovered that alone Hepatitis C is causing Liver cirrhosis and Hepatocellular carcinoma.

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